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This symposium on extracellular microbial polysaccharides of practical importance would not be complete without consideration of the α-D-glucans, dextran and pullulan.

The significance of dextran in man's practical affairs was apparent before its origin, identity, and name were established. Dextrans develop naturally in sucrose-containing solutions that have become inoculated with dextran-producing bacteria from air, plants, or soil. The resulting transformation of the solutions to syrupy, viscous, or ropey fluids, or even to gelled masses, doubtlessly has plagued man since the inception of accumulating and storing sucrose-containing foods and beverages. As early as 1813 (1,*2*)[1/], reports described the mysterious thickening or solidification of cane and beet sugar juices, and later impediment of filtration and crystallization was traced to the occurrence of this condition. In 1861, Louis Pasteur (3) initiated systematic scientific progress by explaining that these "viscous fermentations" resulted from microbial action. In 1878, van Tieghem (4) named the causative bacteria Leuconostoc mesenteroides because its growth in colorless flocs resembled that of the green algae of the genus Nostoc. In 1880 (5), Scheibler established this type of product as a glucan having positive optical rotation and named it dextran.

Thus, through the importance of the dextran class of α-D-glucans in man's economy, the dextrans were the first extracellular microbial polysaccharides to come under systematic scientific investigation. Dextrans from several bacterial strains also were the first extracellular microbial polysaccharides to be produced and used industrially. A comprehensive review (6) was published in 1966 on dextran production,

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[1/] References other than reviews, which cite original research publications not included here, are marked with an asterisk.

structure, properties, uses, and related considerations. The same topics were reviewed from a different viewpoint in 1973 (7). The biosynthesis and structure of dextrans was reviewed comprehensively in 1974 as well as the structurally dependent specific interactions with immunoglobulins (antibodies) and globulins such as concanavalin A (8). An extensive bibliography on all scientific aspects of dextran (exclusive of clinical research and testing) and dextran derivatives includes information from 1861 through mid-1976 (9).

Summarized here is the current status of dextran as an established product of world commerce and in relation to specific industries.

Interest in pullulan and its practical potentialities have developed since 1959 when Bender, Lehman, and Wallenfels (10) first characterized the water-soluble extracellular product from Aureobasidium (Pullularia) pullulans and named it accordingly. The polysaccharide had been isolated previously and partially characterized in studies of microorganisms responsible for breakdown of forest litter (11). The slime-forming black yeastlike fungus, A. pullulans, occurs ubiquitously in organic waste matter which it decomposes in soil, rivers, paper-mill effluents, and sewage (12). The microorganism has adverse economic importance because of its costly deterioration of paint, discoloration of lumber, and attack on plants and plant products (13). In none of these natural occurrences, however, does the polysaccharide seem to have a role except as a slimy nuisance. Already of applied practically, however, is the enzyme pullulanase (pullulan 6-glucanohydrolase EC 3.2.1.41) which was discovered by Bender and Wallenfels in Aerobacter aerogenes (14) and shown to depolymerize pullulan to its repeating unit maltotriose by specific attack on the interunit α-1,6-linkages. Pullulanase, now obtainable in practical amounts from numerous microbial sources, also cleaves α-1,6-linkages in starch and is used industrially to release the unit chains in starch (15,16). Substrates other than pullulan, however, may be used for producing pullulanase.

The production, properties, and potential uses of pullulan have been reviewed (12). Summarized here are the constitutional bases for practical applications and the uses that have been proposed.

Importance of Naturally Occurring Dextrans

The predilection of Leuconostocs for sucrose in nature has a specific basis. Sucrose induces in these bacteria formation of the dextran-synthesizing enzyme dextransucrase (sucrose: 1,6-α-D-glucan 6-α-glucosyltransferase, E.C. 2.4.1.5). This enzyme accomplishes dextran synthesis by

transferase action without need for intermediate substrates. Fructose, the byproduct of dextran synthesis, is metabolized by Leuconostocs which cannot, however, metabolize either sucrose (they have neither invertase nor sucrose phosphorylase) or dextran. Extracellular dextransucrase is produced abundantly by many strains, although the dextransucrase of some strains is cell-bound. The rate and extent of activity on sucrose that may result is illustrated dramatically by an historic report of the fortuitous conversion of 5000 liters of molasses to a compact gel mass in 12 hours (4).

In 1972, the status of the situation was that, "although it is difficult to quantify the effects of polysaccharides on the economics of sugar cane processing, it is obvious from the volume of recent literature that importance is attached to their elimination from the process" (17). The dextrans have a major role (9,17) although bacterial levans and polysaccharides of plant origin are involved also (17). The long-known adverse effects of dextran continue in polarization measurements, clarification and filtration, and in reducing the rate and efficiency of crystallization. In addition, traces of dextran cause inferior crystal structure by elongating the c axis (18,19). The beet sugar industry is less affected by dextran contamination. Sucrose is less exposed to infection during harvesting and first stages of processing of beets than of cane.

Very sensitive biochemical tests have demonstrated the extent of dextran contamination in commercial sucrose, including that distributed as a standard of highest purity. Neill, Hehre, and coworkers (20) demonstrated serological activity indicative of dextran in both cane and beet sugars from diverse geographical sources and various methods of manufacture. The majority of the cane products showed higher serological activity than did the majority of the beet products. The weakest activities were in several samples of reagent grade sucrose of German origin prepared from beet sugar. Gibbons and Fitzgerald (21), utilizing the agglutinizing action of dextran on cells of Streptococcus mutans, also demonstrated dextran in reagent-grade sucrose.

Dextranases are being investigated (22) and used (23) for removal of dextran from cane sugar juices as well as from sucrose solutions and wines made hazy by the presence of dextran.

Constitutional Basis for Practical Importance

Dextran. By definition, the generic name dextran applies to a large class of α-D-glucans in which predominance of α-1,6-linkages is the common feature. One of the simplest dextrans known is that from Leuconostoc mesenteroides NRRL B-512(F); the structural features are shown in Figure 1.

The α-D-glucopyranosidic linkages are 95% 1,6-and 5% 1,3- (24). The 1,3-linkages are points of attachment of side chains of which about 85% are 1 or 2 glucose residues in length (25). The remaining 15% of the side chains may have an average length of 33% glucose residues and may not be uniformly distributed in the macromolecule (26). This dextran is readily soluble in water; certain other dextrans may be insoluble. In dextrans from other strains, the non-1,6-linkages may be 1,2-, 1,3-, or 1,4-. Only one type may occur in a dextran, or there may be two or three. Great diversity is thus created. Dextran available in the United States and western Europe is produced from sucrose by strain NRRL B-512(F). Dextrans of apparently similar structure, but from different strains, are produced in Japan (27) and Russia (28). Dextrans produced in other countries of Europe and Asia are from selected strains (29*).

Dextran having this structure (Figure 1) was selected for production because the fraction ($\overline{M}_w$ 75,000 ± 25,000) prepared from it for intravenous administration (blood volume expander) was substantially less antigenic as compared with that from dextrans having higher percentages of non-1,6-linkages. Dextran from strain NRRL B-512(F) is completely metabolized in man (30) when either ingested or administered parenterally as a fraction of suitable molecular size and size distribution. Derivatization, however, slows or inhibits metabolism.

An additional asset of strain NRRL B-512(F) is its copious formation of dextransucrase (31). Production of dextran by use of cell-free culture filtrates rather than in growing cultures results in enhanced yield, quality and ease of purification of the product. And furthermore, by suitable adjustment of conditions, the major product can be synthesized directly within a chosen molecular weight range (32, 9).

The native dextran may have weight-average molecular weight ($\overline{M}_w$) values (33) (light scattering) of 35-50 X 10^6. The structural simplicity of this dextran permits graded partial depolymerization and separation into fractions of any desired $\overline{M}_w$ and size distribution, which differ primarily in molecular weight. The content of branch points remaining, however, would depend on the method of partial depolymerization; it is decreased by acid hydrolysis (34) but not by use of endo-acting dextranases (1,6-α-D-glucan 6-glucanohydrolase, E.C. 3.2.1.11). Fractions of lower molecular weight obtained through such enzymolysis retain the branch points, and their structural details would be determined by the action pattern of the specific dextranase (35). The series of fractions produced from partial depolymerizates is unique. Selected fractions or derivatives of them serve pharmaceutical or other purposes having specific requirements for molecular

size in order to achieve physiological compatibility or other special objectives.

Production of such fractions by direct, controlled enzymatic synthesis is not known to be in use.

The high proportion of 1,6-linkages in dextran NRRL B-512(F) confers unusual flexibility on the chain and leaves numerous sites for substitution, essentially all of which are in secondary positions. The ratio of relative rate constants established for methylation of the hydroxyl groups, $C_2:C_3:C_4$:8:1:3.5 (36) indicates also the relative reactivity towards other substituents such as the sulfate (37). The frequent occurrence of three hydroxyl groups in consecutive positions in the glucopyranosidic residues of dextrans would appear to account for their unusual ability to complex with large amounts of metallic elements such as ferric iron and calcium. Such complexes are important as pharmaceutical preparations and in certain metallurgical processes.

Thus, the charcteristics of dextran from strain NRRL B-512(F) that determine its value in practical applications, reside in its composition as a soluble α-D-glucan and in the properties of its primary structure. In contrast, it has been emphasized in this symposium that the unique characteristics of the anionic heteropolysaccharide xanthan which are basic to its usefulness, result from secondary and tertiary structural effects (38,39,40). The specific role of ionic charge, which also may be influential in xanthan properties, has not been established but may be inferred from research on ionogenic derivatives of dextran (41).

Pullulan. The generic name pullulan is applied to any extracellular α-D-glucan elaborated by A. pullulans from a variety of substrates. A commonly observed feature is the predominant repeat unit maltotriose polymerized linearly through 1,6-linkages (Figure 2). Frequently present also are α-maltotetraose units (42,43,44) contained mainly within the polymer chain (43) in amounts of 6.6% (43) and 5-7% (44). In products in which possible heterogeneity was not excluded, traces of other neutral sugars and uronic acids have been reported (12). Products from other strains and from other genera and species have shown variation on the basic pullulan pattern such as the presence of 1,3-linked glucosyl residues (45,46). Thus, "there is, perhaps, no unique structure of pullulan" (43).

The molecular weight of a pullulan product differs with the length of fermentation time (47,48). Molecular weight of 2×10^6 developed initially during limited fermentation decreased to 1.5×10^5 during continued fermentation (47). The site of degradation is the internally located maltotetraose units; the degradative enzyme appears to be an "endoamylase" produced during culture growth (43,47). The modified

pullulan resulting from "endoamylase" action is inert to α-amylase (43).

Such uncontrolled variation in molecular weight can be eliminated, however, by choice of strain and adjustment of the pH and of the phosphate content of the culture medium (49). Pullulan products having molecular weights as high as 250×10^4 or as low as 5×10^4 may be obtained in this way.

The mechanism of biosynthesis of pullulan discourages consideration of enzymatic synthesis as a means for production. Synthesis is accomplished through mediation of sugar nucleotide/lipoid carrier intermediates associated with cell membrane fractions (50).

The pullulan molecule may be considered as a chain of amylose, the linear component of starch, in which an α-1,6-bond replaces every third α-1,4-bond. The 1,6-bond introduces flexibility, and the interrruption of regularity results in making pullulan readily soluble, eliminating retrogradation and improving rather than impairing fiber- and film-forming ability. The presence of the 1,6-bonds may influence the position of substituents and properties of derivatives by introducing a different sequence of free hydroxyl groups. The presence of 1,6-linkages, spaced as they are, prevents attack by salivary and intestinal amylases (43). Isoamylase from Pseudomonas sp., which cleaves α-1,6-bonds in amylopectin and glycogen, also is inert on pullulan (51).

Dextran and Dextran Derivatives in Industry

Pharmaceutical Industry. Probably the largest outlet for dextran and dextran derivatives is through the pharmaceutical and fine chemicals industries. The major developments have originated from fundamental research in Sweden which was initiated about 1944 and has continued consistently (52,53). Research and development have followed, however, in numerous other countries throughout the world which produce their own pharmaceutical products from dextran (9,27,28,29*).

Two dextran fractions of major significance are used in suitably prepared solutions for parenteral administration (6,9). The fraction of $\overline{M}_w$ 70,000 is used to restore and maintain blood volume in treatment of shock, hemorrhage, extensive burns, and a variety of other physiological conditions. The fraction of $\overline{M}_w$ 40,000 is used to improve flow in capillaries, treatment of vascular occlusion, artificial extracorporeal perfusion of organs, and in a variety of other ways.

These and other sharply cut dextran fractions are used for preparation of numerous derivatives such as the sulfates, diethylaminoethyl (DEAE) dextran, and complexes with iron and other metallic elements. These substances serve a variety of purposes (9,54,55). Dextran sulfates have anticoagulant, antilipemic, and antiulcer activity. They

are used in liquid two-phase separation and concentration of living cells such as those of viruses, blood, tumors, and other tissues. DEAE dextran enhances biological effects of macromolecules and vaccines. A soluble complex of dextran and iron is produced widely in numerous countries for intramuscular administration to alleviate iron-deficiency anemia in the human and in domestic animals. The solution contains 5% iron and 20% dextran of $\bar{M}_w$ 5,000 (56). The iron is mainly nonionic (56) and appears to be β-FeOOH (57). The initial patents (58) have been emulated extensively (9). A soluble calcium complex containing 10-12% calcium is administered parenterally to alleviate hypocalcemia of cattle delivery paresis (9). Complexes with antimony and arsenic are effective against tropical infections (9).

Crosslinked dextran gels or their ionic derivatives are employed in purification, fractionation and isolation of enzymes, hormones, and other sensitive biological substances without modification of their activity. By covalent bonding to either dextran or crosslinked dextran gels, enzymes, immunoglobulins, and antigens are stabilized and supported for use in specific reactions (59,9).

Dextranases, prepared by growth of various molds on dextrans, are used in mouthwashes and toothpaste to either disperse or inhibit formation of dento-bacterial plaques which contain dextrans and foster carious dental lesions (9,60,61,62).

Food Industry. The potentialities for dextran in the food industry have been reviewed (63,64). The only actual uses known to the author, however, are in dextran gel-filtration processes to concentrate proteins or to recover proteins from liquid wastes and effluent streams. From cereal waste streams, 70% recovery of protein has been effected. In the milk industry, skim milk or cheese whey is fractionated for recovery of undenatured protein components of enhanced quality, nutritive value, and applicability. A plant having capacity of 1×10^6 lb. per day is in operation (65). Protein is separated from lactose and mineral constituents and fractionated mainly on the basis of molecular weight into casein, β-lactoglobulin, and α-lactoglobulin. β-Lactoglobulin, which is highly superior nutritionally to casein (66), had restricted use when previously isolated as the degraded and denatured lactalbumin (67). Other valuable products that may be recovered are lactoferrin and immunoglobulins.

By another application of gel filtration, the protein content of milk is increased from 3.35% to 5.35% without increase of the lactose and mineral contents (68).

Atomic Fuel and Metallurgy. Gel precipitation is a process in which dextran (or certain other polyhydric polymers)

is used to produce a metal compound in the form of a gel under conditions where an insoluble precipitate would be expected (69) (Figure 3). The process is used for purifying, separating, and concentrating metals from solutions of their salts or mixtures of salts or from colloidal dispersions of aqueous hydrous sols. The final product may be in the form of powder, granules, spheres, rods or shaped rods, or ceramic coatings and moulded objects. Products prepared by use of dextran as the gelling agent are for use as nuclear reactor fuels (70,71), catalysts (71,72,73), ceramic coatings (70), refractories and ferro-electric materials (72,73), and powder for alloys (72), pigments (74) and metallurgical processes (70).

The gelling agent and metal ions appear to form molecular complexes which, when contacted with the precipitating reagent, produce discrete microcrystalline gel particles (69,75). The 1,6-linkages in dextran are believed to confer a special configuration on the three contiguous free -OH groups which is peculiarly favorable to -OH--complex formation with an unusual number of metal ions (69,76).

The specific properties of dextran metallic ion complexes are utilized in separating ferric iron from mixtures with copper, nickel or cobalt, or nickel from thorium, or zirconium from copper (76). Dextran (or fractions of stated molecular weight) is utilized in preparing black magnetic iron oxide (Fe_3O_4) from ferrous salt (74,77). Cupric ion may be adsorbed from solution on hydrous gels of ferric oxide, chromium oxide, or thorium phosphate/dextran gel, and then eluted (77).

The procedures reviewed here indicate the potential for dextran application in gel-precipitation processes. Some of the procedures are known to be in use.

Petroleum Production. A pioneering concept advocated for some years was to make dextran a profitable byproduct of the sugar cane industry by using it in petroleum drilling muds (78). In initial laboratory testing for water loss inhibition, a modified dextran gave results equivalent or superior to starch and carboxymethyl cellulose (79). The modified dextran (Viscoba), prepared by treatment of dextran with aldehyde before isolation (80), had improved viscosity and was resistant to microbial attack. The concept was advanced further when, during 1956 through 1959, a dextran production pilot plant was operated in conjunction with a sugar mill in Cuba (81). The dextran, produced from strain NRRL B-512(F) by a modified enzymatic procedure, was precipitated once and drum dried. [The fructose byproduct was recovered and uses investigated (81)]. The output (3-6 tons/day) was used in the United States in drilling muds under a variety of field conditions. The price at the well site was 46 cents/lb.; the demand greatly exceeded the supply (82). The dextran

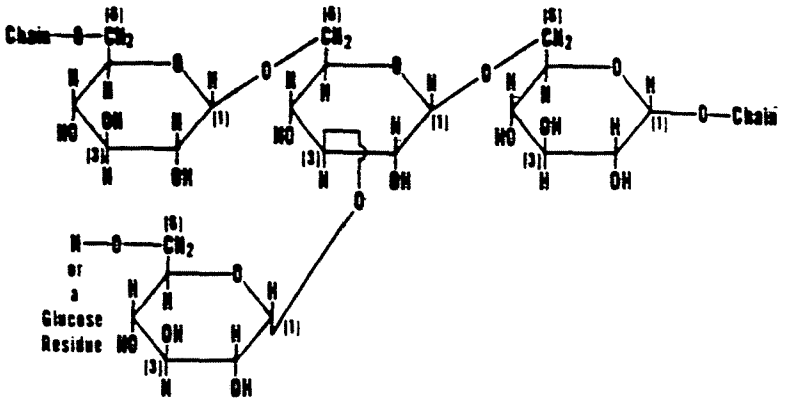

Figure 1. Structural features of dextran from Leuconostoc mesenteroides *NRRL B-512(F)*

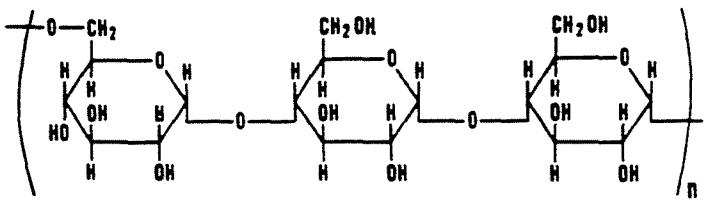

Figure 2. The characteristic structural features of pullulans: α-maltotriose polymerized through α-1,6-linkages

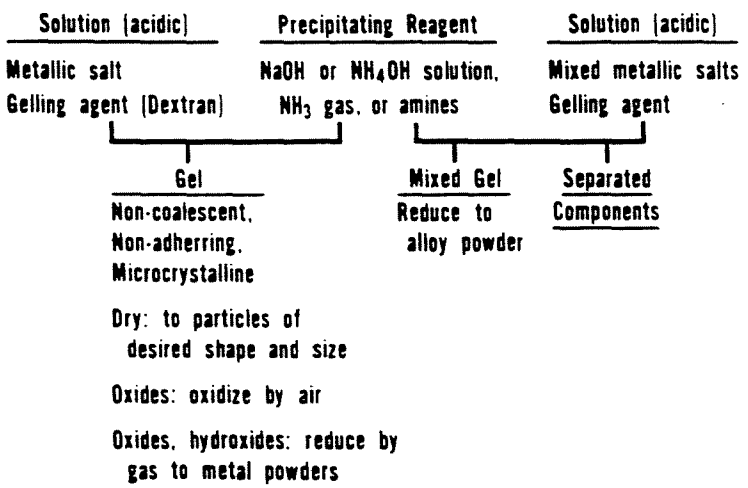

Figure 3. The gel-precipitation process and some of the products resulting

functioned better than some of its competitors and as well as any.[2/]

The dextran could not be used in lime base muds; it precipitated at pH 11.0-11.2 and lost its water-binding capacity. Under these conditions, however, lightly hydroxyethylated dextran retained its water-binding capacity (82). At less basic or neutral pH, dextran tolerates calcium ion and magnesium ion well (83). Dextran is the hydrocolloid in an "inhibited mud" composition containing 3500 ppm calcium ion that is used to inhibit shale hydration (84). Like all polymers, dextran is susceptible to free radical degradation, and protection is advised during processing as well as use (85).

Dextran NRRL B-512(F) has properties suitable for use in viscous water flooding (86); in screening tests, it gave results superior to many other substances examined. The unfavorable results of a field trial (87) may have related to lack of protection against free-radical degradation.

Photographic Industry. Native high molecular weight dextran has been supplied consistently for an undisclosed industrial use believed to relate to photographic products. Numerous patents have been issued in the past and continue to be issued on the superior effects achieved from certain dextran derivatives in X-ray and photographic emulsions (9,54,55). It seems probable that some of these derivatives are in use.

Pullulan--Proposed Uses

Numerous applications of pullulan and its derivatives have been proposed and patented, but apparently are not yet in use (88,89). The possibility of eventual success for most uses is increased by the claim that, by proper selection of pullulan-producing strains, the molecular weight can be controlled and absence of black pigment in the product can be assured (49).

The efficiency of pullulan, even as the crude fermentation liquors, has been demonstrated for flocculation of clay slimes from aqueous solutions resulting from beneficiation of uranium, potash, and other ores (90,91,92).

Films formed from pullulan without plasticizers have excellent physical properties, are water-soluble, impervious to oxygen and suitable for coating or packaging foods and pharmaceuticals especially when exclusion of oxygen is desirable (88). Fibers from pullulan have a shiny gloss and high tensile strength which, after stretching, is described

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[2/] Death of the key personnel in an airplane accident terminated this development.

as comparable to that of nylon (88). The fibers may be admixed with natural fibers in special papers and other products (93). Pullulan is suitable for making adhesives and shaped articles by compression molding. In such molded articles pullulan has characteristics similar to polyvinyl alcohol or to styrene (88). It has desirable properties for use in noncaloric and other foods; it is nontoxic and non-digestible (88). Pullulan is biodegradable, however, under usual conditions of waste disposal.

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